Automated Generation of Focused Gradient Profiles in Preparative Liquid Chromatography

Sample purification using the Agilent 1260 Infinity automated LC/MS purification system

Technical Overview

Authors

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Abstract

With the Agilent 1260 Infinity automated LC/MS purification system, the purification process has reached a new level of automation. A tailored-gradient profile for each target compound was generated to increase the resolution, increase column load, and reduce the cycle runtime. The Agilent automated purification software automatically generated the different tailored-gradient profiles.

This Technical Overview describes an automated purification process of a compound mixture, and shows cross-platform compatibility between different analytical LC systems and the Agilent 1260 Infinity automated LC/MS purification system. A standard mix was analyzed using four different analytical (U)HPLC systems, using different gradient profiles and different column dimensions. The four analytical result files were used to calculate gradient profiles, and purify target compounds. Through the use of this intelligent software algorithm, we obtained automated tailored-gradient profiles for all four samples using the purification process. We purified 100 mg of a sample mix containing 24 mg of the target compound. We obtained 90 % recovery and 99 % purity of the target compound after purification.

Agilent Technologies

Introduction

The Agilent 1260 Infinity automated LC/MS purification system is an easy-to-use, intelligent solution to automate the purification process of compounds after their synthesis. The process starts with an analytical scouting run followed by a nonlinear scale up process that generates a focused gradient profile to isolate dedicated target compounds[1]. The aim of using focused gradient profiles is to increase:

- The chromatographic resolution
- The injection volume
- The efficiency of the overall process by reducing the cycle runtime[2]

Streamlining workflows by defining user privileges

The Agilent automated purification software guides users, with varying levels of chromatographic knowledge, to achieve successful target compound isolation in a highly streamlined process. Sample submission and software features can be adjusted according to the user's expertise level. Two different user modes are available: the Expert mode for the experienced chromatographer, and the Easy-Prep mode for the less experienced user. By selecting the Expert mode, a chromatographer can create template files that contain all parameters required for the purification process. Required parameters are for example, the system dwell volumes, extra column volumes, column dimensions, and customized focused gradient profiles, which have been created from an experienced chromatographer to purify different compound libraries showing specific chromatographic behaviors.

When switching to the Easy-Prep mode, the user can choose from a list of predefined templates covering their needs, then start the purification process. Table 1 list the different user privileges.

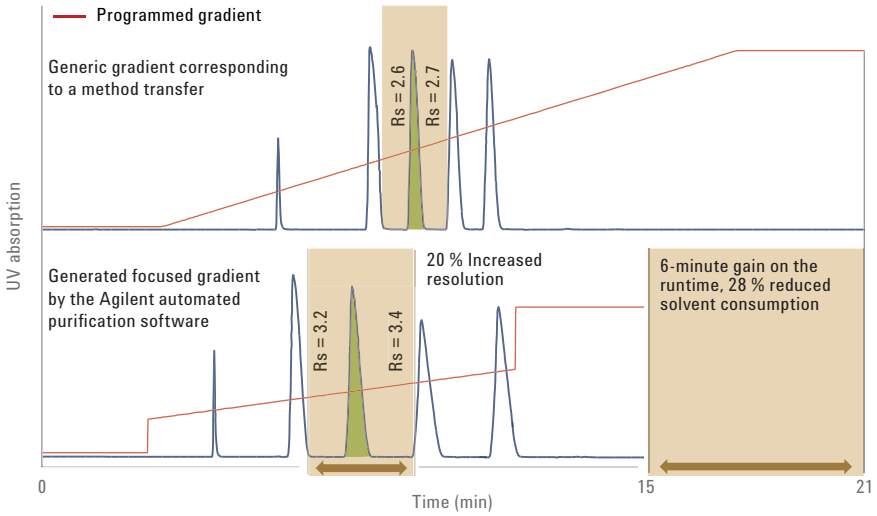

Figure 1. Comparison of two chromatograms showing identical samples. The lower chromatogram shows an increase of resolution and a reduced runtime after a focused gradient profile has been applied to the target compound (highlighted in green).

Table 1. User privileges for the two different user modes.

	Expert users	Easy-prep users
Create a new or template task	✓	
Use a template for a task submission	✓	✓
Change systems, and columns task characteristics	✓	
Review or not the analytical data	✓	✓ If allowed in the template
Assign a different target peak	✓	✓
Change gradient profile	✓	

This Technical Overview describes the automated purification process of a compound mixture, and demonstrates a cross-platform compatibility between different analytical HPLC and UHPLC instrumentation and the purification system. A standard mix was analyzed using four different analytical LC systems with different gradient profiles and column dimensions. The analytical result files were used to calculate tailored-gradient profiles, and purify the target compounds.

Experimental

Instrument

Analytical systems

- Agilent 1290 Infinity binary LC/MS system
- Agilent 1260 Infinity binary LC/MS system
- Agilent 1220 Infinity LC system

Table 2 lists the individual components of an Agilent 1260 Infinity automated LC/MS purification system.

Columns

- Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm (857700-902)
- Agilent ZORBAX SB-C18, 4.6 × 50 mm, 1.8 µm (827975-902)
- Agilent ZORBAX SB-C18 Rapid Resolution, 4.6 × 100 mm, 3.5 µm (861953-902)
- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (883975-902)
- Agilent ZORBAX Prep HT SB-C18, 21.2 × 150 mm, 5 µm (870150-902) with end fittings (820400-901)

Software

- Agilent OpenLAB CDS A.01.05 (ChemStation Edition) with ChemStation C.01.05 HF05
- Add-on OpenLAB CDS Automated Purification Software A.01.01 for Agilent OpenLAB CDS ChemStation Edition

Solvents and samples

Solvent A

Water + 0.1 % formic acid

Solvent B

Acetonitrile + 0.1 % formic acid

All solvents were LC grade, not degassed. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak)

Sample mixture for the preparative task

Caffeine (5 mg/mL) eluted as peak 1, methyl-4-hydroxy-benzoate (37.5 mg/mL) as peak 2, ethyl-4-hydroxy-benzoate (37.5 mg/mL) as peak 3, propyl-4-hydroxy-benzoate (37.5 mg/mL) as peak 4, and benzyl-4-hydroxy-benzoate (37.5 mg/mL) as peak 5 (Figure 2). All compounds were dissolved in DMSO.

Chromatographic and spectrometric conditions

Table 2. Components of Agilent 1260 Infinity automated LC/MS purification system.

No.	Description	Part number
1	Agilent 1260 Infinity preparative pumps	G1361A, G1391A
2	Agilent 1260 Infinity dual-loop autosampler	G2258A
3	Agilent 1260 Infinity isocratic pump	G1310B
4	Agilent 1260 Infinity diode array detector	G1315C
5	Quartz flow cell 10-mm path length	G1315C#018
6	Agilent 1200 Series column organizer	G1383A
7	Agilent 1260 Infinity preparative-scale fraction collector	G1364B
8	Agilent active splitter	G1968D
9	Agilent 6100 Series single quadrupole mass spectrometer	G6130B

Table 3. Analytical generic method parameters and flow rates: See chromatograms (Figure 2).

Parameter	Value
Analytical injection volume and flow rates	Agilent 1290 Infinity binary LC/MS system: 1 µL, Flow: 1 mL/min Agilent 1260 Infinity binary LC/MS system: 1.7 µL, Flow: 1.75 mL/min Agilent 1220 Infinity LC system: 3.3 µL, Flow: 1.75 mL/min Agilent 1260 Infinity automated LC/MS purification system: 5 µL, Flow: 1,75 mL/min
UV wavelength (analytical and prep)	280 nm, 70 nm bandwidth, no reference
Analytical gradient profiles	Figure 2 displays the analytical gradient profiles.
Automated focused preparative gradient	Generated automatically based on results of analytical scouting run. Target compound identification was from molecular formula/mass.
Preparative flow rate	30 mL/min
Injection volume	650 µL

Table 4. Mass spectrometric conditions.

Parameter	Value
Scan/Fragmentation parameters	
Ionization mode	API-ES, Positive/Negative switching
Percent cycle time/polarity	50.0 %
Scan mass range (negative and positive)	125–725
Fragmentor	70 V
Gain EMV	1.0
Threshold	150
Step-size	0.20
MSD spray chamber	
Gas temperature	350 °C
Drying gas	12.0 L/min
Nebulizer pressure	50 psig
Quadrupole temperature	350 °C
VCap	Positive: 3,100 V Negative: 3,000 V

Results and Discussion

Analytical results

The standard mix was injected into four different chromatographic systems. Figure 2 shows the results, injection volumes, column dimensions, and gradient profiles.

The target compound is highlighted in all chromatograms, and was identified by the software after processing the mass spectrometry data. All chromatograms have different run times. In each chromatogram, the target compound elutes at a different retention time.

Purification process

Using the four different analytical systems, the Agilent automated purification software is programmed to upload and process the different analytical data files, and purify the samples that have been analyzed.

The set up starts by entering the properties of the fluidics and the column dimensions of the analytical systems. The properties of the preparative system and column dimensions have to be entered, and all experimental parameters are saved as data files.

Possible combinations of the four analytical systems with the preparative system were selected and saved as different template files. During the data processing step, the target compounds were identified in each analytical chromatogram by mass spectrometry. Tailored focused preparative gradients were created automatically, and were used to improve the system's purification efficiency (Figure 3).

A user interface displays the properties of the fluidics from a selected analytical and preparative system combination (Figure 4).

To simplify the data file transfer, the analytical HPLC and UHPLC systems are linked with the purification system by a network connection. Analytical data files are uploaded to the Agilent automated purification software. After an analytical result file has been processed, adjustments can be made to optimize gradient profiles, and correct vial locations, injection volumes, and target masses (Figure 5).

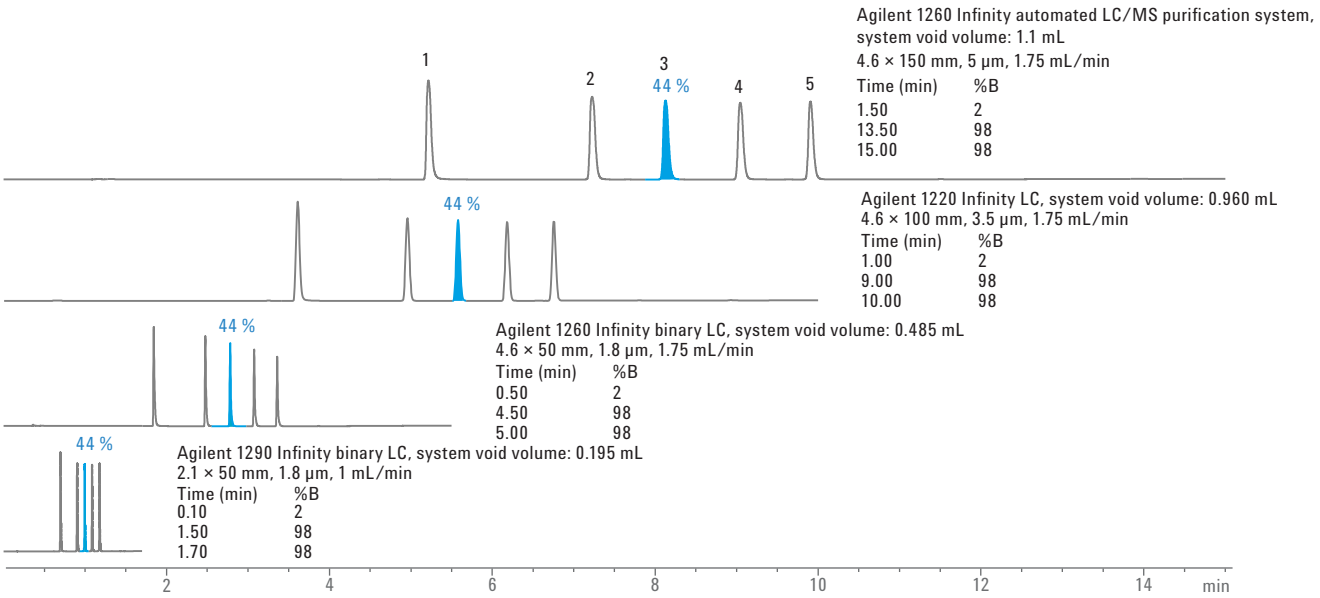

Figure 2. Results acquired on the four different analytical systems LC systems. The elution sequence is: peak 1: caffeine, peak 2: methyl-4-hydroxy-benzoate, peak 3: ethyl-4-hydroxy-benzoate, peak 4: propyl-4-hydroxy-benzoate, and peak 5: benzyl-4-hydroxy-benzoate.

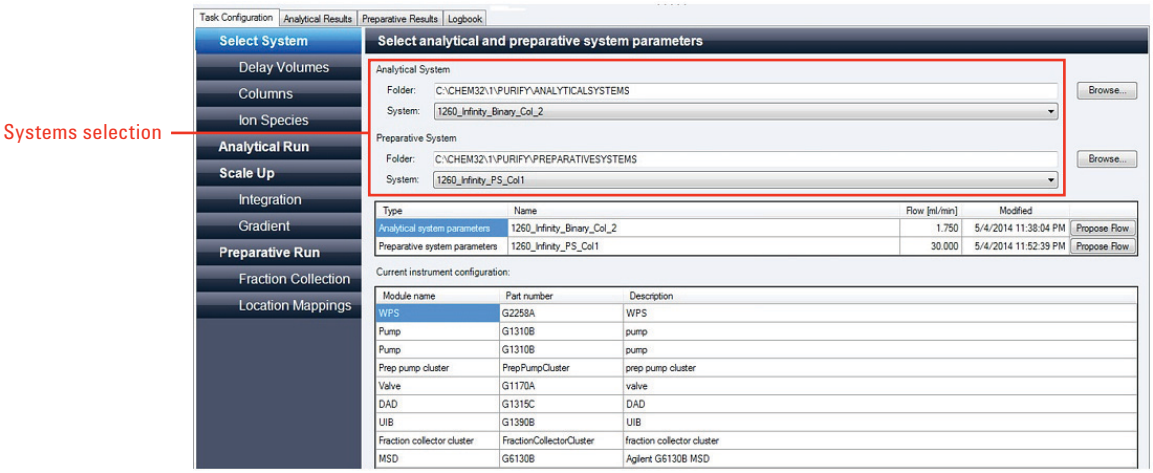

Figure 3. System selection pane: Possible combinations of analytical and preparative systems are selected.

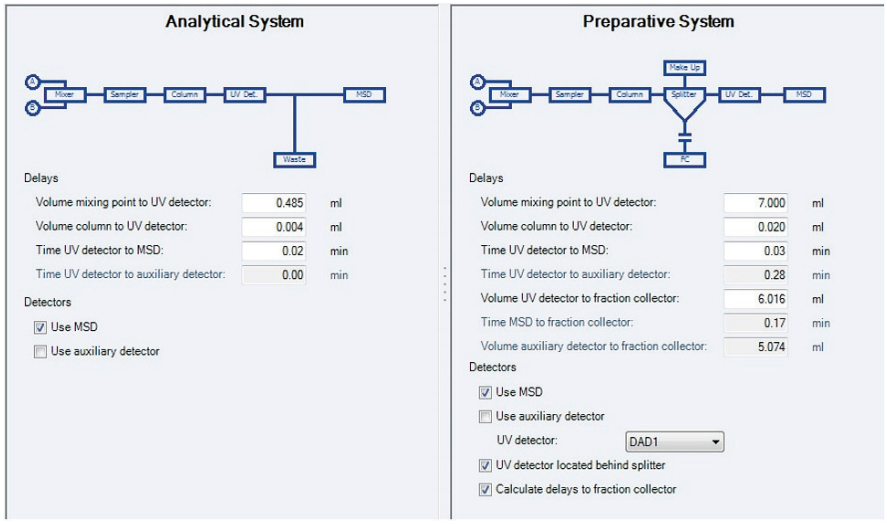

Figure 4. Overview window indicates the systems parameters.

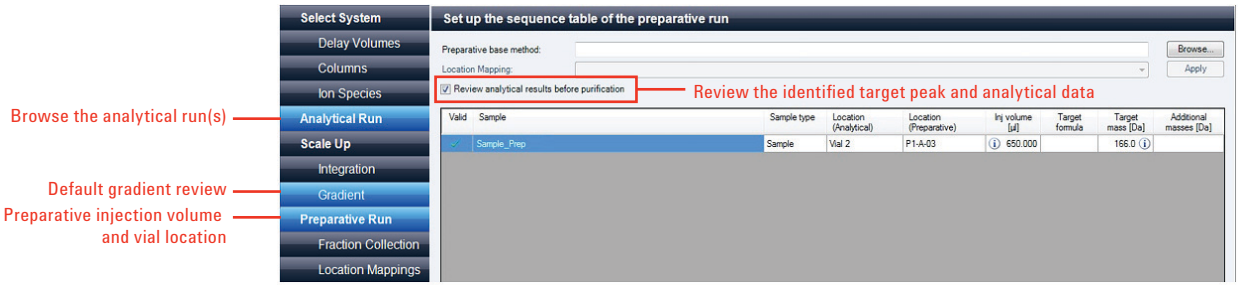

Figure 5. Uploaded analytical results, reviewing and modifying vial locations, target masses and injection volumes.

To demonstrate the automated purification capabilities of the software using different analytical systems, all four analytical result files were uploaded and processed (Figure 6).

The algorithm calculates the percentage of organic solvent used to elute the target compound during the analytical run. Even when using different column dimensions, flow rates, and the gradient profiles mentioned above[3], the percentage of organic solvent in the mobile phase to elute the compounds remains constant, within a tolerance of 2 %. The algorithm of the Agilent automated purification software calculates the focused gradient profile based on the percent of organic solvent needed for the elution and is, therefore, independent from the retention times of the compounds. The Agilent automated purification software will generate valid focused gradient profiles no matter which analytical system was used to acquire the data.

The elution points of all compounds of the test mix were listed and compared. All elution points were within a tolerance of 2 % (Table 5).

After processing the analytical data files, the analytical results browser in Figure 7 shows the identified target peak (highlighted), and shows all acquired spectral data information at a glance.

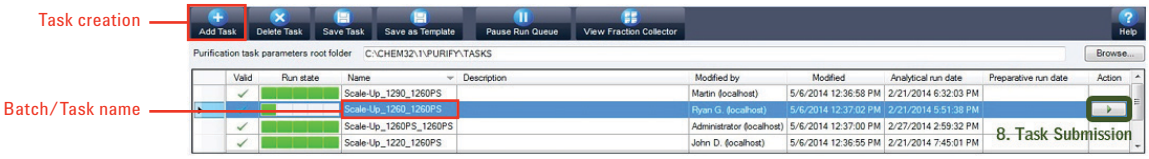

Figure 6. Task creation window showing uploaded and processed data files.

Table 5. Elution points of test compounds on different analytical LC systems.

	Column	Flow rate (mL/min)	Ep(1) %	Ep(2) %	Ep(3) %	Ep(4) %	Ep(5) %
Agilent 1290 dw = 0.2 mL	2.1 × 50 mm, 1.8 µm	1	23	37	44	49	56
Agilent 1260, dw = 0.485 mL	4.6 × 50 mm, 1.8 µm	1.75	22	37	44	51	58
Agilent 1220, dw = 0.96 mL	4.6 × 100 mm, 3.5 µm	1.75	21	37	44	51	58
Agilent 1260*, dw = 1.1 mL	4.6 × 150 mm, 5 µm	1.75	21	37	44	52	58

* Agilent 1260 Infinity automated LC/MS purification system

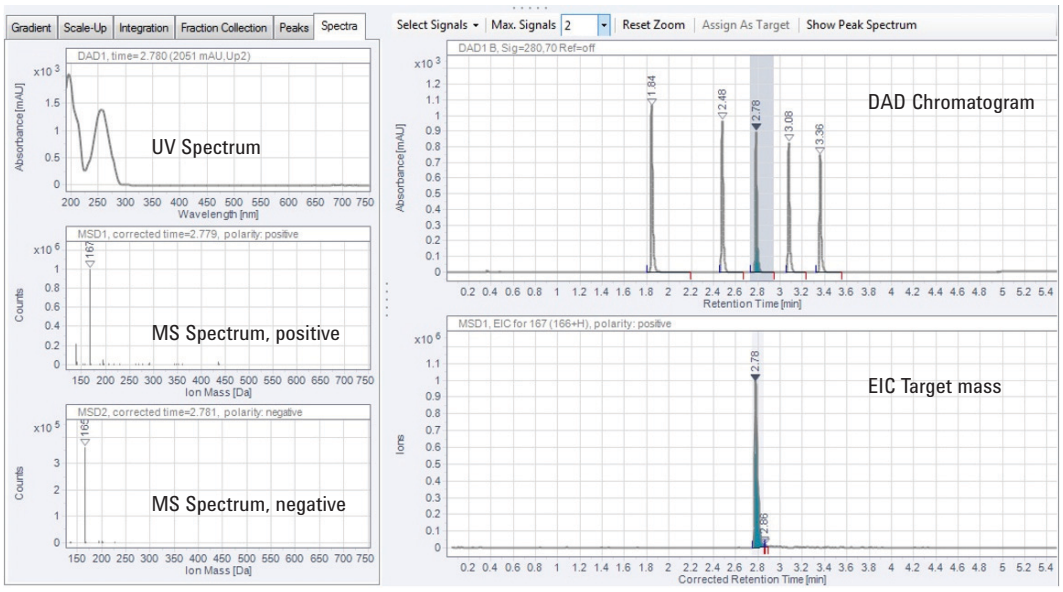

Figure 7. Analytical results browser showing all acquired chromatographic and spectral data at a glance.

Focused Gradient Profiles

An important feature of the Agilent automated purification software is the graphical display of the focused gradient profile (Figure 8). In this view, all relevant gradient parameters can be adjusted for individual tasks.

The steepness of the focused gradient profile is defined by the offsets and the slope duration. The offsets of start and end points have been defined by default to –15 % below and +5 % above the elution point of the selected compound.

All calculated offsets, slopes, flow rates, and isocratic and purge steps can be modified by the chromatographer (expert user) and saved as a template file.

The data process delivered consistent elution points of the target compound for all acquired data files. An automated purification process can be supported, even if the analytical data files have been created using different HPLC and UHPLC systems, flow rates, or column dimensions. To prove the concept, a preparative injection was initiated to purify the targeted compound. A 650-µL test mix solution dissolved in DMSO with a total compound content of 100 mg was injected onto the preparative column.

Smart trigger algorithm

The software automatically selects the best trigger source for fraction collection. A compound with a low UV absorption will be collected automatically by MS signal only. Conversely, a compound with poor ionization electrospray characteristics will be automatically triggered only by UV absorption. If both signals are available, the most intense above threshold level will be selected, and the peak trigger will be a combination of both detectors linked by the Boolean *and* logic.

Preparative results browser

After completion of the preparative run, the purification results can be viewed using the Preparative Results tab (Figure 9). In the task configuration and results section, the following information is displayed:

- Vial location, injection volume, and target mass submitted for the mass-based fraction collection

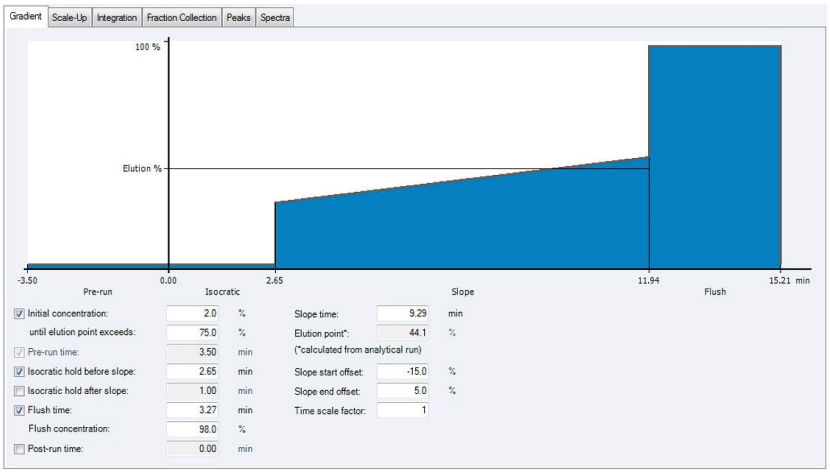

Figure 8. Gradient profile UI, all listed parameters can be modified by the user.

Figure 9. Preparative results with 650 µL injection volume on an Agilent ZORBAX Prep HT SB-C18, 21.2 × 150 mm, 5 µm column.

- Collected fractions on the fraction collector diagram, or fraction data using the fraction table tab. Locations, collected volumes, the main ion of the collected compound (*m/z*), start and stop collection times, as well as the type of collection (triggering UV or MS)
- By selecting a fraction on the chromatogram, the average spectral data will be displayed on the right side of the chromatogram.

The fraction table information can be exported as a csv, Excel, or text file to a liquid handler for fraction pooling, or for database import. For fraction re-analysis, a sequence file can be created automatically and exported to an Agilent analytical LC/MS system or by using the capabilites of the Agilent 1260 Infinity automated LC/MS purification system.

Conclusion

This Technical Overview shows the analysis of a crude sample using four different HPLC and UHPLC systems with different column dimensions, flow rates, and gradient lengths. All analytical data files were processed using Agilent automated purification software to generate tailored focused gradient profiles. To demonstrate the methodology, a preparative injection was performed to purify the target compound. In this Technical Overview, 22 mg of the target compound, corresponding to 90 % of recovery, were recovered. After fraction re-analysis, a purity level of 99 % purity was obtained. A streamlined and automated purification process is supported, even when the analytical result files have been acquired on different HPLC and UHPLC systems.

References

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